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EFFECT OF BENZYLAMINOPURINE (BAP) AND MICROPLANT POPULATION ON MICROTUBERISATION IN POTATO (*SOLANUM TUBEROSUM* L.)

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Abstract

Two experiments were conducted to produce microtuber in a potato cv. Diamant using two levels of BAP (5.0 and 7.5 mg/l) with a fixed dose of sucrose (8%). Three plant population (10, 20 and 30) and three age group of microplants (20, 30 and 40 days) were used in the experiments. The maximum number of microtubers and weight of microtubers per flask were 26.17 and 3432.31 mg when 30 plants were cultured with 7.5 mg/l BAP. The mean weight of each microtuber was the highest for 5 mg/l BAP and 10 plant population (215.50 mg) against the minimum of 129.11 mg with the same BAP level and 30 plant population. The application of BAP @ 5.0 mg/l with 10 plant population produced the maximum effective size (100-200 mg plus >200 mg) and microtuber (87.05%). Twenty days old microplants with 7.5 mg/l BA produced the maximum number of microtubers and weight of microtubers per flask (7.33 and 1332.0 mg respectively). The maximum mean weight was 190.4 mg with 5 mg/l BAP and 20 days old microplants compared to the minimum 124.7 mg with 7.5 mg/l BAP and 40 days old microplants. BAP @ 5 mg/l with 20 days old microplants produced the maximum effective size (100-200 mg plus >200 mg) and microtuber (83.57%). The maximum desirable size microtuber (>100 mg) could be obtained by using 10 plantlets per flask with 5 mg/l BAP (87.05%) or 20 days old plantlets with 5 mg/l BAP (83.57%).

Introduction

Potato is a cash crop in Bangladesh. Seed health is one of the important factors for development of this sector. Virus free seed potato tuber is the major concern. Production of seed potato is a highly technical matter. Few years back, clonal system was followed for the production of seed potato. With the development of tissue culture technology, the only method of producing virus-free seed potatoes, the clonal system has been replaced. At present about 38 tissue culture laboratories are in operation in the country and most of the laboratories are producing seed potatoes with microplants. None of them producing microtubers in spite of lot of advantages (easy to handle, store, planting, etc) due to lack of suitable protocols. Microtubers are produced *in vitro* on complete plantlets or on plant organs by changing the nutrient media and /or the external conditions. *In vitro* produced microtubers are generally weight to 0.2 g per tuber or less (Hussey and Stacey, 1984; Garner and Blake, 1989). Usually a whole microplant produced one microtuber, occasionally two.

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A number of factors like photoperiod, light intensity, temperature, nitrogen level in media, debudding, sucrose concentration, physiological age of microplants in association with growth promotors or retardants (Menzel 1981; Leclerc *et al.,* 1994; Dobranszki and Mandi, 1993; KabSeog, 2004) are responsible for *in vitro* tuberisation. Among the growth promotors, BAP stimulates microtuberisation very effectively (Hussey and Stacey, 1984). Generally, 5.0 to 10 mg/l BAP are used for microtuber production in potato, though 2.0 mg/l gave comparative better yield performance to 5 mg/l BAP. However, the production of microtubers can be accelerated by modifying the plant population in culture. According to CIP protocol (Tovar, 1985), 30 single nodes are usually used in a 250 ml Erlenmeyer flask in order to harvest 27 microtubers but the average size was not promising. On the other hand, age of microplants is also an important factor which influenced the production of microtubers in potato. Hence, the study was undertaken to enhance microtuber production of the potato cv. Diamant using different factors like BAP, plant population and age of microplants.

Materials and Methods

The *in vitro* ready stocks of microplants of the potato cv. Diamant (Dutch origin) were used in two seperate experiment. In the first experiment, two levels of benzylaminopurine (BAP) at 5.0 and 7.5 mg/l and three levels of plant population (10, 20 and 30 nodal cuttings per 200 ml flask) and in the second experiment the above two levels of BAP and three age group of microplants (20, 30 and 40 days old) were used. In the second experiment, five microplants of 5-6 cm long excluding the roots were used in each 200 ml flask. In both the experiments the plantlets were developed *in vitro* through liquid culture as described by Estrada *et al.* (1986). The culture media were replaced to tuberisation media having MS basic salts supplemented with BAP as per treatments, 8% sucrose and in the second experiment, liquid culture media for growth of microplants were replaced in tuberisation media as per treatments.

Media composition for plantlet growth in liquid shaken culture media was as described by Dodds *et al.* (1988). The pH of all media was adjusted to 5.8 and the media were autoclaved at 15 psi and 121^{0}C temperature for 20 minutes. Forty millilitre culture medium was poured in each culture jar. The culture jars for plantlet growth were incubated under 3000 lux light intensity for 16 hrs daily and at 22^{0}C temperature. Tuber formation was done under complete dark condition having a temperature of 19±1^{0}C. The experiment was laid out in a complete randomised design (CRD). The microtubers were harvested 90 days after the addition of tuberisation media. Hussey and Stacey (1984) harvested microtuber 12-15 weeks after the addition of tuberisation media. The microtubers were harvested under the laminar air flow cabinet and the individual tuber weight as per treatments was measured and the tubers were graded into more than 200, 100-200 and less than 100 mg size by number. Data on microtuber production and yield were recorded, analysed and the means were separated using LSD.

Results and Discussion

Days to tuber initiation: The number of days required for tuber initiation due to BAP level and plant population was not significantly different. However, tuberization was delayed by 7.50 days when 7.5 mg/l BAP was used with 30 plant population where it was earlier by same level of BAP with 20 plant population. It appeared that plant population in relation to BAP did not affect tuberization (Table 1), whereas tuberisation was affected due to age of microplants. The

30 days old microplants cultured with 7.5 mg/l BAP took the minimum of 6.16 days for tuberisation which was statistically similar to 40 days old microplants with 5.0 mg/l (Table 2).

The dominating role of nitrogen in plant growth and tuber formation of potato plant has been emphasised by several workers (Okazawa, 1967; Palmer and Smith, 1970). As regularity factor for tuberisation, its form and concentration are highly significant. But both very low or very high levels of nitrogen are inhibitory, though high level is more deletorius than low level (Garner and Blake, 1989). In the present study, 60 Mm/L the N was used with 5.0 or 7.5 mg/l BAP which supplied the required amount of nitrogen and thus did not affect initiation of microtubers in culture. However, for tuber initiation it required exogenous supply of nitrogen coupled with different stimulating agents like, IAA, 2, 4-D, NAA, BAP, etc. According to Hussey and Stacey (1984), Leclerc *et al.* (1994) and Shibli *et al.* (2001) that tuber initiation and growth in potato is control by a balance between inhibitors and promoting substances, which is indicative to antigibberellins like cycocil, coumarin, abscisic acid, jasmonoid, cytokinin, triiodobenzoic acid, meleic hydrazide, etc. (Choi *et al.*, 1993; Paet and Zamora, 1994; Hossain and Sultana, 1998; Silva *et al.*, 2001).

No. of tubers per flask: The number of microtubers per culture jar varied significantly due to plant population in flask and BAP levels. It ranged from 6.16-26.17. The number of tuber per flask increased with increasing plant population. BAP at 5 mg/l with population density of 10 produced only 6.16 microtubers per flask which increased to 25.33 at plant density of 30. Similarly, 7.5 mg/l BAP with plant population of 10 produced only 6.83 microtuber, which increased to 26.17 at plant density 30 (Table 1). The number of tubers per plants did not vary significantly due to age of micro plants. which ranged from 5.0-7.33. The younger plants (20 days old) produced the maximum microtubers per flask which gradually decreased with increasing age of microplants. The 30 days old microplants cultured in 50 mg/l BAP had the minimum number of microtubers per flask (5.00) closely followed by 40 days old microplants with 7.5 mg/l BAP (Table 2).

Plant population is considered as an important factor in producing more number of microtubers from a single culture jar as indicated by Jimenez *et al.* (1999). In general, more the number of plant in culture, more the number of microtubers. However, Hossain and Sultana (1995) obtained heavier microtubers from lower plant population, though high plant population increasing the number of microtubers, which was in agreement with the findings of the present investigation. Most of the workers used different plant population for improving microtuber production programme. But Khomyak *et al.* (1998) used different plant population with different volume of culture media to improve the microtuber production while Forti *et al.* (1992) used single node explants at different plant densities for microtuberisation in a better way.

Weight of microtuber per flask: The weight of microtuber per flask differed significantly due to plant density and BAP levels used in the culture media (Table 1). The highest weight of microtuber per flask was 3132.31 mg/l with 7.5 mg/l BAP and a plant population of 30 which was statistically similar to 3205.21 mg with 5.0 mg/l BAP and a plant population of 30 (Table 1). The trend was almost similar for population density of 10 and 20 with 5.0 and 7.5 mg/l BAP, respectively. The maximum weight was 1332 mg for 20 days old microplants cultured in 5 mg/l BAP which was statistically similar to 7.5 mg/l BAP (1288.0 mg). The minimum weight of

microtuber was 662.00 mg with 40 days microplant in 7.5 mg/l BAP. The weight of microtuber decreased gradually with increasing age of microtuber (Table 2). It indicates that younger microplants are more suitable for microtuber production.

The physiological age of the mother tuber act on tuberization either directly or indirectly mediating changes in hormone concentrations (Vander Zaag and Van Loon, 1987; Burton, 1989; Ewing and Struik, 1992). Most authors agree that the yield of physiologically older seed is markedly higher than that of younger seed if harvested prematurely (Bus and Schepers, 1978; Bean and Allen, 1980). But several authors have found lower yields with physiologically old seed than with young seed (Reust, 1982). Other researchers found almost no effect of physiological age on yield (Bus and Schepers, 1978). Moll (1985) found that the yield of early cultivars grown from old seed was lower than their yield when grown from young seed. The physiological age of mother tubers used as a source of material influenced kinetin-induced *in vitro* tuberization. Tuberization significantly increased with physiologically young plant (Villafranca *et al.*, 1998). Tuber inducing activity was detected in leaves and old tubers using single-node and stem. Akita and Takayama (1988) found greatest microtuber by using *in vitro* plantlet of 4 weeks old in continuous dark. Similar result was observed by Rosell *et al.* (1987).

Mean weight of each microtuber: The mean weight of microtuber varied significantly due to the treatment combination of population density and BAP levels. The lower the plant population the higher the mean weight of microtuber per flask. The maximum mean weight of each microtuber was 215.50 mg under 5.0 mg/l BAP and a plant population of 10 per flask which reduced gradually to 129.11 mg with a plant density of 30 (Table 1). Similarly, the mean weight of microtuber under 7.5 mg/l BAP with a plant population of 10 was 200.21 mg which gradually reduced to 132.81 mg under a plant population of 30 (Table 1). The mean weight of each microtuber decreased gradually with microplant age. However, the maximum was 190.40 mg for 20 days old microplants cultured with 50 mg/l BAP while the minimum was 124.7 mg for 40 days old microplants with 7.5 mg/l BAP (Table 2).

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Tuber grading

The size distribution of microtuber in number percentage of >200 mg varied significantly due to combined treatment of BAP levels and plant population (Table 2). The maximum percentage of > 200 mg size microtuber was 55.18 under 5.0 mg/l BAP with a plant density of

10 which was statistically similar to 48.89% under 7.5 mg/l BAP with the same population (Table 1). On the other hand, the maximum percentage of >200 mg size microtuber in relation to BAP levels and plant age was 49.82 under 5.0 mg/l BAP and 20 days old microplants which was statistically similar to 7.5 mg/l BAP levels and 20 days old microplants (45.11%). The minimum percentage of >200 mg size microtuber was produced when 7.5 mg/l BAP was used in culture media to produce microtuber in 40 days old microplants (28.89) (Table 2).

Table 1. Combined effect of BAP and plant population on the production and size of microtubers of potato cv. Diamant

Treatment		Days to tuber initiation	No. of microtuber per flask	Wt of microtuber per flask (mg)	Mean micro tuber wt. (mg)	Grades of tubers (mg) by no. (%)		
BAP level (mg/l)	Plant population					>200	100-200	<100
5.0	10	6.66	6.16	1319.02	215.50	55.18	31.87	12.96
	20	6.66	14.50	2541.21	178.12	48.75	26.77	23.30
	30	7.00	25.33	3205.21	129.11	23.07	41.83	35.09
7.5	10	6.50	6.83	1351.03	200.21	48.89	26.75	26.75
	20	6.00	16.11	2535.12	159.81	33.22	40.62	26.16
	30	7.50	26.17	3432.31	132.81	19.67	43.40	36.94
LSD 0.05		ns	4.32	546.12	25.18	6.60	7.26	8.48

ns = non-significant

Table 2. Combined effect of BAP and plant age on the production and size of microtuber of the potato cv. Diamant

Treatment		Days to tuber induction	No. of micro tubers per flask	Wt. of micro tuber per flask (mg)	Mean micro tuber wt. (mg)	Grades of microtuber (mg) by no. (%)		
BAP level (mg/l)	Plant age (day)					>200	100-200	<100
5.0	20	6.33	7.00	1332.00	190.40	49.82	33.75	16.43
	30	11.83	5.00	813.30	164.80	33.93	40.48	25.6
	40	7.83	5.66	890.20	157.40	41.03	35.87	23.1
7.5	20	7.33	7.33	1288.00	174.90	45.11	29.76	25.13
	30	6.16	6.66	1094.00	165.00	42.38	33.06	24.56
	40	11.33	5.33	662.00	124.70	28.89	34.17	36.94
LSD 0.05		1.83	1.20	205.60	17.16	6.52	5.00	9.73

In case of 100-200 mg size microtuber, it was almost reverse to >200 mg size grade except 5.0 mg/l BAP and plant population of 10 and 20 and 7.5 mg/l BAP and plant population 10. The minimum percentage of microtuber was 26.75 under 7.5 mg/l BAP and plant population of 10 and the maximum was 43.40 under 7.5 mg/l BAP and a plant population of 30. The maximum percentage of effective size microtuber (>200 mg plus 100-200 mg) was 87.05 when 5.0 mg/l BAP was used to produce microtuber in 10 microplants per flask.

Regarding <100 mg size microtuber irrespective of BAP levels (5.0 or 7.5 mg/l) the percentage of microtuber increased with increasing plant age. It was 12.96% under 5.0 mg/l BAP and plant population of 10 which increased to 35.09% at plant population of 30 with 7.5 mg/l BAP. It was 26.75% with 7.5 mg/l BAP and plant population of 10 which increased to 36.94 with plant population of 30 (Table 2). The treatment combination of 5 mg/l BAP and plant age of 30 days produced the maximum percentage of 100-200 mg size microtuber

(40.48%), closely followed by the same BAP level and a plant age of 40 days (35.87%). The minimum percentage of 100-200 size microtuber was 29.76 when 7.5 mg/l BAP was used in culture media to produce microtuber in 20 days old microplant. Irrespective of BAP levels 20 day old microplants produced minimum percentages of 100-200 mg size microtuber which gradually increased with increasing plant ages. The maximum percentage of effective size microtuber (>200 mg plus 100-200 mg) was produced by 5.0 mg/l BAP and 20 days old microplant (83.57%).

The combined treatment of 7.5 mg/l BAP and 40 day plant age produced the maximum percentage of <100 mg size microtuber (36.94%) and the minimum was 16.43% under 5.0 mg/l BAP and 20 day plant age. The other combined treatments varied in between 23.10-25.60%. At both the levels of 5.0 and 7.5 mg/l BAP, the 20 and 30 day plant ages produced almost similar percentages of microtuber (Table 2).

Conclusion

From the above results it may be concluded that culture of 10 plantlets per flask with 5 mg/l BAP (87.05%) or 20 days old plantlets with 5 mg/l BAP (83.57%) for microtuber production could give the maximum desirable size microtuber (>100 mg).

References

Akita, M. and S. Takayama. 1988. Mass propagation of potato tubers using jar fermentor techniques. *Acta Hort.*, **130:** 55-61.

Bean, J.N. and E. J. Allen. 1980. Effect of physiological age and variety on growth light interception in the potato. *Pot. Res.*, **23**: 256-257.

Burton, W. G. 1989. Potato varieties, breeding and propagation. In: The Potato (W.G.Burton ed.), 3rd edition, London. pp. 47-50.

Bus, C.B. and A. Schepers. 1978. Influence of pretreatment and physiological age of seed on growth and yield of potatoes cv. Bintje. Proceedings 7th triennial conference of European Assoc. for Pot. Res., (Warsaw). pp. 16-17.

Catchpole, A.H. and J. Hillman. 1969. Effect of ethylene on tuber initiation in *Solanum tuberosum* L. *Nature* (London), **223**: 1387.

Choi, Y.W. J.L. Cho and L.S.Kim. 1994. Studies on rapid *in vitro* multiplication of potato (*Solanum tuberosum* L.) microtubers and their practical use and dormancy of microtubers. *J. Korean Soc. Hort. Sci.*, **35**:213-219.

Dobranszki, J. and M. Mandi. 1993. Induction of *in vitro* tuberisation by short day period and dark treatment of potato shoots grown on hormone-free medium. *Acta. Biol., Hungary*. **44**:411-420.

Dodds, J.H., P.Tovar, R.Chandra, D.Estrella and R.Cabello. 1988. Improved methods for *in vitro* tuber induction and use of *in* vitro tubers in seed programs. In: Proc. Symp. on Improved Potato Planting Material, Asian Potato Assoc., kunming, China, June 21-24, 1988. pp. 157-158.

Estrada, R., P.Tovar and J.H. Dodds, 1986. Induction of in vitro tubers in a broad range of potato genotypes. Plant Cell Tiss. *Org. Cult.*, **7**: 3-10

Ewing, E.E. and P.C. Struik. 1992. Tuber formation in potato: Induction, initiation and growth. *Hort. Rev.*, **14**: 89-198.

Forti, E., G.Mandolino and P.Ranalli. 1991. *In vitro* tuber induction: Influence of the variety and of the media. *Acta Hort.*, **300**: 127-132.

Garner, N. and J.Blake, 1989. The induction and development of potato microtubers *in vitro* on media free of growth regulating substances. *Ann. Bot.*, **63**: 663-674.

Hossain, M.J. and N. Sultana. 1995. Report on tuber crops tissue culture of 1994-95. Presented in the internal review of TCRC during 15-17, July, 1995 at BARI, pp.1-16.

Hussey, G. and N.J. Stacey. 1984. Factors affecting the formation of *in vitro* tubers of potato (*Solanum tuberosum* L.). *Ann. Bot.*, **53**:565-578.

Jimenez, E., N. Perez, M. de Feria, R. Barbon, A. Capote, M. Chavez, E. Quiala, J.C.Perez and M. de Feria. 1999. Improved production of potato microtubers using a temporary immersion system. *Plant Cell Tiss. Org. Cult.*, **59**:19-23

KabSeog, Y., D.W.M. Leung and K.S.,Yoon. 2004. Relative importance of maltose and sucrose supplied during a 2-step potato microtuberisation process. *Acta Physiol. Plantarum*, **26**:47-52.

Khomyak, M.V.; P.A. Melnik and V.V.Khomyak. 1998. A laboratory study on accelerated multiplication of virus-free potato material. Paper presented in EPPO conference on potato proptection, Ukraine, 7-10 July, 1998. Bulletin OEPP., **28**:573-577.

Leclerc, Y., D.J. Donnely and J.E.A. Seabrook. 1994. Microtuberization of layered shoots and nodal cuttings of potato: the influence of growth regulators and incubation periods. *Plant Cell Tiss. Org. Cult.*, **37**:113-120.

Menzel, C.M. 1981. Tuberization in potato at high temperature: Promotion by disbudding. *Ann. Bot.*, **47**:727-733.

Moll, A. 1985. Due einfluss des physiologischen alters der pflanzknollen auf die ertragsbildung von kartoffelsorten verschiedener reifezeit. *Potato Res.*, **28**:223-250. (Cited from CAB abstr.).

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plantarum,* **15**:473-497.

Obata-Sasamoto, H. and H. Suzuki. 1979. Activities of enzymes relating to starch synthesis and endogenous levels of growth regulators during tuberization of isolated potato stolons cultured *in vitro*. Z. *Pflanzenphysoil*. **95**:69-75.

Okazawa, Y. 1967. Physiological studies on the tuberisation of potato plants. J. Fac. Agric., Hokkaido Univ., **55**:267-336.

Palmer, C.E. and O. E Smith. 1970. Effect of kinetin on tuber formation on isolated stolons of (*Solanum tuberosum* L.) Cultured *in vitro*. *Plant Cell physiol.*, **11**: 303-314.

Paet, C.N. and A.B.Zamora. 1994. Production of *in vitro* tuberlets and greenhouse plantings. Proc. of the 4th Triennial conf. of the Asian Potato Assoc., 5-8 May, Seoul, South Korea. pp.17-19.

Pelacho, A.M. and A.M. Mingo-Castel, 1991. Jasmonic acid induces tuberization of potato stolons cultured *in vitro*. *Plant physiol.*, **97**: 1253-1255.

Reust, W. 1982. Contributional appreciation delage physiologique des tubercules de pomme de et etude de son importance sur le rendement. These No. 7046, presentee al Ecole polytechnique Federale. Aurich. 113pp. (Cited from CAB abstr.)

Rosell, G., F.G. Bertoldi and R. Tizio. 1987. *in vitro* mass tuberization as a contribution to potato micropropagation. *Potato Res.*, **30**: 111-116.

Simko, I. 1993. Effects of kinetin, paclobutrazol and their interactions on the microtuberisation of potato stem segments cultured *in vitro* in the light. *J. Plant Growth Reg.*, **12**:23-27.

Shibli, R.A., A.M. Abu-Ein and M.M. Ajlouni. 2001. *In vitro* and *in vivo* multiplication of virus-free 'Spunta' potato. *Pak. J. Bot.*, **33**:35-41.

Tovar, P., R. Estrada, L. Schilde-Rentschler and J.H. Dodds. 1985. Induction of *in vitro* potato tubers. *CIP Circular.* **13**:1-4.

Van der Zaag D.E. and C.D. Van Loon. 1987. Effect of physiological age on growth vigour of seed potatoes of two cultivars. 5. Review of literature and integration of some experimental results. *Potato Res.*, **30**:451-472.

Villafranca, M.J.,V.S. Veramendi and A.M. Mingo-Castel. 1998. Effect of physiological age of mother tuber and number of subcultures on in vitro tuberisation of potato. *Plant Cell Reports*, **17**:787-790.